Decomposition of nucleic acids in soil

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Abstract

Mineralization of carbon (C) and nitrogen (N) from yeast RNA and herring sperm DNA were measured in soils and sands for up to 130 days. Nucleic acids were degraded rapidly in neutral sand and silt loam soils with approximately 78% of nucleic acid-N becoming mineralized after 30 days whereas after this time in an acidic (pH = 4.6) sandy silt loam soil, only 7% of nucleic acid-N was mineralized. The mineralization of nucleic acid-C occurred at a lower rate than N mineralization in all soils. At the end of the decomposition experiment, similar amounts of N were mineralized from DNA and RNA in sand samples (78% and 80% respectively), although less C was mineralized from DNA than from RNA.

Keywords: DNA - RNA - persistence - soil - carbon mineralization - nitrogen mineralization.

Introduction

There has been renewed interest in assessing the persistence of nucleic acids in soils since the large scale release of genetically modified organisms. Concern over the potential for transfer of transgenic DNA to indigenous micro-organisms has led to methods designed to detect transgenic DNA in soil. Many studies have investigated nucleic acid interactions with specific soil fractions, but few have focused on the decomposition of nucleic acids in whole soils. Nucleic acids can be released into the soil environment from many sources including dead plant and microbial cells, or by excretion from viable microbial cells. Early studies showed that pure nucleic acids in soil microcosms were quickly digested by nucleases with the release of inorganic phosphorus (P) (Greaves & Wilson 1970). However, despite the presence of numerous DNase-producing organisms in soil, DNA has been found to persist in agricultural soils for extended periods of time, as demonstrated by PCR amplification of target DNA fragments (Widmer *et al.* 1997; Hay *et al.* 2002). Many studies have shown that DNA bound

to soil components such as clay can persist in soil for extended periods because the bound DNA is less accessible to enzymatic degradation (Paget *et al.* 1992). Nucleic acids can be bound to clay and sand particles, rendering them more resistant to microbial nucleases (Lorenz & Wackernagel 1992).

Little work has been published on decomposition of nucleic acids in soil. The majority of studies have focused on tracking specific gene sequences using PCR-based techniques (Widmer *et al.* 1997; Paget *et al.* 1998) rather than measuring the appearance of by-products of nucleic acid decomposition e.g. inorganic N and P. Upon introduction to soil, nucleic acids would be expected to be decomposed by soil microbes to produce carbon dioxide, mineral N and inorganic P (Swift *et al.* 1979). The amounts of such by-products appearing during decomposition and measured over time should provide information on the likelihood for transgenic DNA to persist and be available for uptake by soil bacteria. Greaves & Wilson (1970) showed the rapid breakdown of organic P in nucleic acids to inorganic P in a variety of soil types with soil pH greater than 5.4 in short term (seven day) experiments, whereas the breakdown of nucleic acids in soils with pH 4.6 was very slow.

The objective of this study was to determine the mineralization of N and C from soils differing in texture and pH containing added DNA and RNA. Such a study would be expected to complement the popular PCR strategies designed to detect short oligonucleotides originating from the degradation of functional genes in ecosystems.

Table 1. Some properties of soils used in nucleic acid decomposition experiments.[1]

Soil	Sand	Ignited alluvial sand	Temuka silt loam	Atarau sandy silt loam
Collection site and land use	Beach, New Brighton, Canterbury, NZ	Riverbed, Waimakariri, Canterbury, NZ	Permanent pasture, no fertiliser Lincoln, Canterbury, NZ	Beech forest, Atarau, Westland, NZ
Total N (%)	0.26	0.001	0.25	0.25
Initial mineral ($NH_4^+ + NO_3$)-N (ppm)	4	1	22	12
Organic matter content (%)	7.4	0	4.7	9.0
pH – initial	7.1	9.0	6.0	4.6
Soil particle size analysis	100% Sand	100% Sand	4% Sand 28% Clay 68% Silt	52% Sand 17% Clay 31% Silt
CEC[2] (me/100g)	2	<1	14	13

[1] Further collection details can be found in Keown (2003).
[2] CEC = Cation Exchange Capacity.

Materials and methods

Single stranded herring sperm DNA and Torula yeast RNA (both from Sigma Chemical Company, USA) were used. The N content of nucleic acids and soils was determined by the Kjeldahl procedure using Hg catalyst and a salt acid ratio of 0.6 as described by Greenfield (1999). The total oxidisable organic C content of the nucleic acids was determined using chromic acid oxidation as described by Hesse (1971).

The soils (Inceptisols, U.S. Soil Classification; Table 1) used in these studies were air-dried, sieved to < 2 mm and stored in airtight containers until required (Keown 2003). Five ± 0.05 g (dry weight) of soil was added to 125 ml Erlenmeyer flasks into which 50 ± 0.2 mg DNA (13.7% N) or RNA (14.4% N) was thoroughly mixed before the addition of distilled water to give 20% by weight of soil, equivalent to 55% of water holding capacity. Each sample was set up in triplicate with corresponding controls (no nucleic acid). Soil inoculum (250 µl of settled soil suspension prepared from 20 g fresh garden soil (pH 6.5) briefly shaken with 60 ml distilled water) was added to each flask. Flasks, completely randomised, were incubated for up to 130 days at 22°C in the dark. Moisture contents were checked weekly by weighing. Three replicate microcosms were destructively sampled and analysed for the presence of soil mineral N at 30-day intervals. Mean values and standard errors (SE) were determined and are presented in Figure 1. Values for mineral N (Bremner 1965) in control soils were subtracted from mineral-N values determined for experimental flasks.

N mineralization values were calculated using the following expression:

$$\% \text{ N min} = \frac{\text{E min-N minus C min-N}}{\text{Total N in added nucleic acid}} \times 100$$

where N min = net N mineralized from added nucleic acid; E min-N = total mineral N present in experimental flasks containing known amount of added nucleic acid N; C min-N = total mineral N present in control flask with no added nucleic acid.

Carbon mineralization experiments consisted of similar microcosms which contained vials holding 2M NaOH to absorb respired carbon dioxide (Terry *et al.* 1979). These flasks were sealed with rubber bungs and the amounts of CO_2 respired was determined by acid titration and recorded weekly until CO_2 production declined. Control CO_2-C values were subtracted from CO_2-C values obtained for experimental flasks. Mean values and SE of these values were determined and are given in Figure 2. In most cases, the SE values were so low, they were obscured by the symbols used on the figures.

C mineralization values were calculated using the following expression:

$$\% \text{ C min} = \frac{\text{E min-C minus C min-C}}{\text{Total C in added nucleic acid}} \times 100$$

where C min = net C mineralized from added nucleic acid; E min-C = total C evolved from experimental flasks containing known amount of added nucleic acid C; C min-C = total carbon evolved from control flask with no added nucleic acid.

Results

Mineralization of N from nucleic acids occurred rapidly in three soils

(Figure 1). Nucleic acid degradation was similar in both sandy soils. Most N mineralization occurred within 30 days, approximately 79% and 76% respectively for RNA and DNA; after 90 days these values were approximately 82% of the total nucleic acid-N. Rapid degradation of RNA occurred in Temuka silt loam, with 74% of RNA-N mineralized over 30 days. In contrast, mineralization of DNA-N was much slower in Atarau sandy silt loam soil where only 7% was mineralized after 30 days, increasing to 42% after 90 days. Increased N mineralization of DNA in Atarau soil was accompanied by an increase in the soil pH, from 4.7 to 6.2 over this time. Between 60-80% of the C in RNA and 45-65% of that in DNA had been mineralized after 130 days (Figure 2).

Discussion

Soils used in these experiments varied in several characteristics including texture and pH, but with the exception of the Atarau soil, nucleic acids were rapidly decomposed under the optimum temperature and moisture conditions used. In Temuka soil (28% clay), nucleic acid-N was mineralized more readily than in soil with a lesser clay content (Atarau soil, 17% clay). Both these soils had a similar cation exchange capacity and total N although their organic matter content and initial soil pH differed. Greaves & Wilson (1969) found that binding of nucleic acid to clay is pH dependent; below pH 5.0, nucleic acids bound to internal clay surfaces while above pH 5.0, adsorption was largely confined to external clay surfaces. During decomposition of nucleic acids the pH in Atarau soil increased from 4.6 to 6.2 between day 30 and day 90, concomitant with the appearance of large amounts of mineral-N. The increase in pH may have caused the release of NH_4^+ ions from clay sites. In contrast, the pH in the Temuka soil was never below 6.0 throughout the

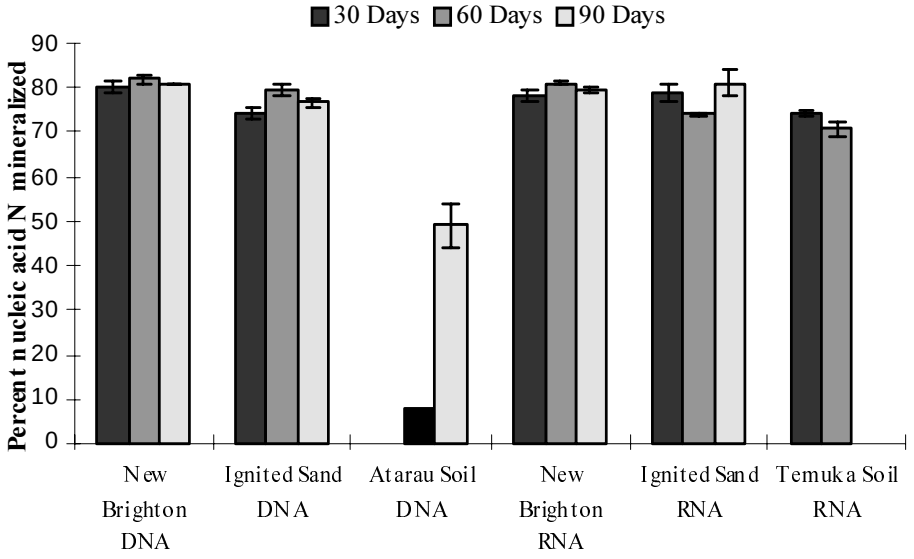

Figure 1. N mineralization (± SE) as a percentage of initial nucleic acid-N from herring sperm DNA and yeast RNA incubated in soil microcosms at 22°C.

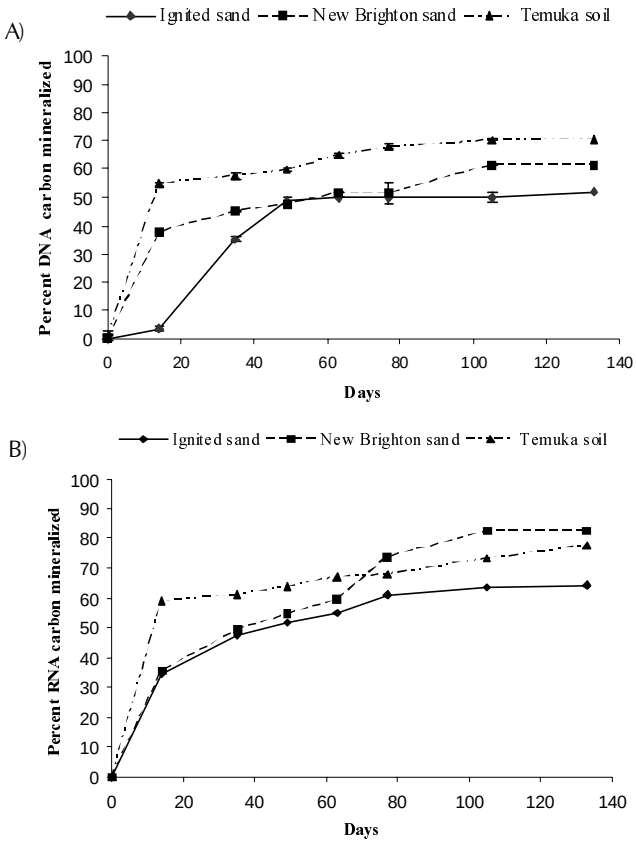

Figure 2. Percentage nucleic acid-C recovered as CO_2-C (± SE) from A) herring sperm DNA and B) yeast RNA incubated in soil microcosms at 22°C.

experiment and the nucleic acids were degraded rapidly.

The rate of nucleic acid decomposition in different soils may depend on many interacting factors, e.g. particle size and pH, together with the quality and quantity of organic matter present in soil and the soil microflora present. Although comparable with the amounts of nucleic acids used by Greaves & Wilson (1970), the amount of nucleic acid used here (10 mg nucleic acid per gram soil) exceeded that found in many soils (0.03 – 1 µg DNA per gram soil; Selenska & Klingmuller 1992; Ogram *et al.* 1987). Although we determined mineralization of elevated levels of nucleic acids in a laboratory study, there was no reason to suggest that similar enzymatic breakdown would be unlikely to occur in the field and that in addition P, N and C mineralization may be used to study nucleic acid breakdown in soils. In this respect it is interesting to note that Bremner & Shaw (1954) found that yeast nucleic acid added to a sandy loam soil (pH 6.1) at 1.6 mg per gram soil decomposed releasing 82% of the initial nucleic acid-N in mineral form over 70 days. This amount is very similar to the values found in this study fifty years later. It confirms that naked or uncomplexed nucleic acid is not

especially resistant to microbial degradation, at least in soils with neutral pH but more work is required in the case of acid soils. From this study, it may be inferred that genetically modified nucleic acids similarly decompose and from those microcosms containing sand, it can be suggested that unaccounted for nucleic acid N and C is likely transformed into the microbial biomass developing during the decomposition of the nucleic acids.

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